Amendments to the Specification :

Please amend the specification by inserting before the first line the sentence:

--This is a 371 of International Application No. PCT/US00/12103, filed 04 May 2000, which claims benefit from the following Provisional Application No. 60/133,391, filed 10 May 1999.--

Please replace the paragraph beginning at page 2, line 28, with the following amended paragraph:

In another aspect, the invention provides a novel method for performing amplifying selected sequences by PCR, which is particularly well suited for use in the stage three of the method of the invention. In this method, a mixture containing the fusion products prepared according to stage 2 of the method of the invention is heated for about 5 minutes in the absence of polymerase or primers at about 94[[EC]]°C, cooled to 50[[EC]]°C over about 30 minutes, at which temperature it is maintained for about 5 minutes or longer. A thermostable polymerase is then added to the mixture, which is heated to about 72[[EC]]°C for about 5 minutes, and mixed with a forward primer P1 for the first region and a reverse primer P4 for the second region. The resulting mixture is then amplified using PCR to produce a modified nucleic acid molecule comprising the first and second regions of the nucleic acid sequence flanking the cassette.

Please delete the following text beginning at page 4, lines 5-8 :

~~**Brief Description of the Drawings**~~

~~Fig. 1 is a flow diagram illustrating the use of the three-stage PCR method of the invention to construct a modified nucleic acid molecule containing a selected cassette.~~

Please delete the following text beginning at page 10, lines 24-26 :

~~For purposes of illustrating three-stage PCR, reference throughout the following discussion may be made to Fig. 1. However, other embodiments of the methods beyond that illustrated in the figure are described below.~~

Please replace the paragraph beginning at page 11, line 1, with the following amended paragraph:

The PCR steps performed in the method of the invention are performed with a thermostable DNA or RNA polymerase and a polymerase having 3'-5' exonuclease activity to remove non-template bases at the 3' and 5' ends. For example, an example of a particularly suitable thermostable DNA polymerase is *Taq* DNA polymerase. The native enzyme may be purified from *Thermus aquiticus* or genetically engineered from the enzyme may be synthesized or obtained from a commercial source (e.g., as ~~AmpliTaq~~™AMPLITAQ®, by Applied Biosystems, which is a 94 kDa ultra-pure, gelatin-free, thermostable, recombinant DNA polymerase obtained by expression of a modified form of the Thermus aquaticus Taq DNA polymerase gene in E. coli). *Taq* is particularly desirable because it carries 5' polymerization-dependent exonuclease activity. Thus, if this polymerase is selected, it is only necessary to include in the reaction mixture a proof-reading polymerase with 3' exonuclease activity. Suitably, high fidelity polymerases are also desirable because they possess 3' and/or 5' exonuclease activity. Examples of high fidelity polymerases include *Pfu* (has 3' proof-reading activity), *Pwu* (has 5' proof-reading activity), *Vent*, *Deep Vent*, *Hot Tub*, *Tfl*, and *Thr* polymerases. However, other suitable polymerases may be selected and obtained from a variety of commercial sources (e.g., Stratagene). Alternatively, other DNA polymerases may be readily selected and 5' and/or 3' exonucleases added if these functions are not provided by the selected polymerase. Such polymerases and exonucleases may be readily selected by one of skill in the art and obtained from a variety of sources. Reaction conditions are as specified by the enzyme supplier with extension times adjusted for the expected product size. See, also, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual", 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), ch. 14.2-14.4 for a general discussion of suitable PCR reagents, buffers, and conditions.

Please replace the paragraph beginning at page 13, line 3, with the following amended paragraph:

Thus, the third amplification ~~stageinvolves~~stage involves the following procedure. A mixture containing the products to be amplified, e.g., the two fusion products obtained from stage 2 amplification, is heated in the absence of polymerase or primers. Suitably, this may be performed in a standard buffering solution, e.g., 50 mM KCl, 10 mM Tris.Cl and 1.5 mM $MgCl_2$. The heating step is performed for about 2 to about 8 minutes, preferably about 5

minutes, to a temperature of about 85[[EC]]°C to about 96[[EC]]°C, and preferably about 94[[EC]]°C. The heated mixture is then taken to a temperature of about 45[[EC]]°C to 55[[EC]]°C, and most preferably about 50[[EC]]°C, over an extended period of time. Most suitably, the cooling takes place over at least about 20 minutes, and preferably over at least 30 minutes. Thereafter, the mixture is maintained at about the same temperature, e.g., at about 50[[EC]]°C, for at least about 5 minutes. However, this temperature may be maintained for a longer period of time such as an hour, several hours, or overnight, if required for convenience.

Please replace the paragraph beginning at page 13, line 16, with the following amended paragraph:

Following this incubation at 50[[EC]]°C, a thermostable polymerase is added to the mixture. A suitable RNA or DNA polymerases may be readily selected. See, discussion of polymerases in section relating to stage 1 amplification. The mixture containing the products for amplification and the polymerase (and exonucleases) are heated to about 55[[EC]]°C to about 75[[EC]]°C for about 3 to about 20 minutes. Preferably, this heating is performed at about 72[[EC]]°C for about 5 minutes. The primer P1 for the upstream region and the primer P4 for the downstream region are then added to the mixture which is subjected to a standard 30 cycles of PCR with an extension time appropriate for the expected full-length product.

Please replace the paragraph beginning at page 14, line 1, with the following amended paragraph:

Optionally, the plates or tubes containing the final product, i.e., modified nucleic acid molecule, may be stored in the freezer (e.g., at -80[[EC]]°C) while awaiting further testing. Where desired, the final product is purified using any of a variety of suitable means, e.g., agarose gel electrophoresis, and, optionally, a sample may be sequenced to confirm the identity of the product.

Please replace the paragraph beginning at page 17, line 22, with the following amended paragraph:

Suitably, the molecules of the invention are combined with one or more pharmaceutically acceptable carriers, for examples, solvents, diluents and the like, and are

administered in the form of sterile injectable solutions or suspensions containing the molecules in an isotonic medium. Generally, the modified nucleic acid molecules of the invention are delivered in an amount of about 0.01 [[□g]]μg to 100 mg per kg body weight. The molecules may be suspended in a carrier, as identified above, and delivered in doses of from about 1 mL to about 30 mL by any suitable route, including, without limitation, intravenous, intramuscular, subcutaneous, and oral. The method of administration is not limited to the delivery routes specified herein. It is within the skill of one in the art to determine the appropriate dosage regimen, taking into consideration such factors as the condition to be treated, the age, weight, sex and condition of the patient, and the like.

Please replace the lines beginning at page 18, line 18 and 19, with the following amended lines 18, 19 and 19.1:

P3 [SEQ ID NO: 3] 5'**GGAAAGTTACACGTTACTAAAGG**CTGGGGCACGCT CATTCTTACA 3'

P4 [SEQ ID NO: 4] 5' TTTTCATAGTGCCTCCAACCTT3'

Please replace the paragraph beginning at page 18, line 24, with the following amended paragraph:

The two-piece PCR reaction was performed using *S. pneumoniae* isolated chromosomal DNA as template. In separate PCR reactions, P1/ P2 were used to produce the upstream region and P3/P4 were used to produce the downstream region using PCR with Taq polymerase ~~[AmpliTaq]~~(e.g., as AMPLITAQ®, by Applied Biosystems, which is a 94 kDa ultra-pure, gelatin-free, thermostable, recombinant DNA polymerase obtained by expression of a modified form of the Thermus aquaticus Taq DNA polymerase gene in E. coli) and Pfu proof-reading polymerase. Reaction conditions were as specified by the enyzme supplier with extension times adjusted for the expected product size. The cassette was produced using a similar PCR reaction. Each final product was purified to homogeniety on an agarose gel column.

Please replace the paragraph beginning at page 20, line 13, with the following amended paragraph:

Stage III was performed by mixing 0.5μg of each the upstream region/cassette and cassette/downstream region in a standard Taq polymerase PCR without ~~polyerase~~polymerase

or primers. The reaction was held for 5 minutes at 94[[EC]]°C, and then taken to 50[[EC]]°C over a ramp period of 30 minutes. The reactions was then held at 50[[EC]]°C for 5 minutes. During this time, 2.5 U of Taq polymerase was added, and the reaction was taken to 72[[EC]]°C for an extension time of 5 minutes. After this period, P1 and P4 are added, and the reaction was subjected to a standard 30 cycle PCR.